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(19) (CA) **CANADIAN PATENT** (12)

(54) PROCESS FOR PRODUCING ETHYL ALCOHOL FROM
AMYLACEOUS RAW STOCK

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PROCESS FOR PRODUCING ETHYL ALCOHOL FROM AMYLACEOUS
RAW STOCK

The present invention relates to the fermentation branch of food industry and is concerned with processes for producing ethyl alcohol from amylaceous raw stock, such as, say, wheat, barley, or rye.

Prior-art processes for producing ethyl alcohol from amylaceous raw stock are known to be based on hydrolysis and fermentation, and to comprise the following stages:

- /1/ crushing the corn grains;
- /2/ rendering the grains pastelike and grain cooking;
- /3/ enzymic hydrolysis of cereal starch;
- /4/ fermenting;
- /5/ wash distilling.

There is extensively applicable currently a process for producing ethyl alcohol from amylaceous raw stock, such as rye, barley, or wheat by hydrolyzing in the presence of malt enzymes. Malt is known to comprise predominantly α - and β -amylases that hydrolyze starch to maltose and glucose, which are then yeast-fermented into alcohol.

The above-discussed process involves, however, top-grade grains for malt preparation, which therefore is a highly critical product. In addition, non-amylaceous substances, such as cellulose, hemicellulose, and some others, contained in grains are not hydrolyzable by the malt enzymes.



Further improvement in the ethyl alcohol production techniques has heretofore been aimed at searching for such microbial-origin enzymic preparations that would be able to hydrolyze starch, hemicellulose, cellulose, and some other substances contained in corn grains.

There have been proposed some processes for producing ethyl alcohol from amylaceous raw stock by hydrolyzing the latter in the presence of amylolytic enzymes produced by mould fungi and bacteria. Such fungial and bacterial enzymic preparations possess more potent complex of enzymes than malt and hence are capable of hydrolyzing the starch-containing grain portion somewhat more completely. On that account the yield of ethyl alcohol per unit of grain raw stock is higher due to the effect of fungial- or bacterial-origin amylolytic enzymes. Substitution of malt for the above enzymic preparations in producing ethyl alcohol enables one to save to a great extent high-grade corn grains which have heretofore been spent in great amounts for malt preparation.

However, the afore-mentioned enzymic preparations obtained from mould fungi and bacteria, can hydrolyze starch only and practically produce no hydrolyzing effect upon non-amylaceous carbohydrates, such as cellulose and hemicellulose, and also some other polysaccharides.

One prior-art process for producing ethyl alcohol is known to consist in hydrolyzing corn grains in the presence

of an enzymic preparation obtained from the grown culture of *Aspergillus niger* /cf. USSR Inventor's Certificate No. 67,012, cl.C 12c 7/04, published on February 20, 1941/. However, raw stock hydrolysis occurs in this case but incompletely, and a great proportion of carbohydrates involved remain unfermented. This is due to the fact that the above preparation fails to contain a required complex of enzymes.

One more prior-art process for producing ethyl alcohol from corn grains is known to involve the use of an enzymic preparation obtained from the grown culture of a mould fungus named *Aspergillus oryzae* /cf. USSR Inventor's Certificate No. 119,852, cl.C 12c 7/04, published in 1958/. However, this preparation fails to improve hydrolysis, too.

An enzymic preparation of phosphatase has been proposed for carrying out more complete hydrolysis of amylaceous raw stock /cf. USSR Inventor's Certificate No. 460,292, cl.C 12c 7/04, published on May 15, 1975/. But even this preparation fails to bring about complete hydrolysis of amylaceous raw stock.

The enzymes listed above are engaged only in hydrolysis of the amylaceous portion of corn grains and produce practically no effect upon such hardly hydrolyzable grain polysaccharides as cellulose and hemicellulose whose hydrolyzates can be converted into ethyl alcohol.

There have also been proposed some processes for producing ethyl alcohol involving the use of enzymes capable of hydrolyzing carbohydrates contained in the grain wall cells. The enzymes mentioned above are produced by *Trichoderma viride* /cf. US Patent No.3,616,220, cl.C 12b 1/00 published on October 26, 1971/, *Aspergillus niger* /cf. Patent of GDR No.77,176, cl.6a22 3/10 published on October 20, 1970/, *Trichotecium roseum* /cf. USSR Inventor's Certificate No.316,719, cl.C 12c 7/04 published on October 7, 1971/. However, the above-mentioned producers fail to generate such an active complex of hydrolytic enzymes that is required for complete and thorough-going hydrolysis of grain raw stock.

A process for saccharifying grain raw stock with a mixture of malt and an enzymic preparation from *Aspergillus awamori* mould fungus containing xylanase and β -glucanase, is protected by USSR Inventor's Certificate No.467,929, cl.C 12c 7/04 published on December 25, 1974. This preparation hydrolyzes xylane and β -glucane, whereby the yield of ethyl alcohol is increased by 2 to 2.5 per cent. However, the application of the above enzymic preparation enables one to substitute malt by 30 to 35 per cent only.

Known in the art are some methods of cellulose processing in the presence of highly purified, concentrated or immobilized enzymes obtained from the cultures of microor-

ganisms grown by the submerged cultivation method, e.g., *Trichoderma viride* culture /cf. US Patent No. 3,642,580, cl.C 12d 13/04 published February 15, 1972/. Canadian Patent No.975,313, cl. 195-16 published on February 23, 1972, discloses a process for treating starch and protein-containing vegetable material with proteolytic enzymes, whereas French Patent No.2,382,497, cl. C 12c 11/12 published on November 3, 1978 deals with a process for producing ethyl alcohol by virtue of cellulose fermenting under the effect of a mixed culture at a temperature of 50 to 65°C and the pH of 7 to 8. However, this patent fails to specify what producers are employed.

It is an essential object of the present invention to search for such an enzymic preparation that would contain the required complex of hydrolytic enzymes capable of hydrolyzing the nonamylaceous portion of the raw stock into fermentable sugars, thereby increasing the yield of ethyl alcohol.

The above object is accomplished due to the provision of a novel enzymic preparation, containing a complex of hydrolytic enzymes produced by a mould fungi *Trichoderma kőningii* which is capable of more complete hydrolysis of amylaceous raw stock.

According to the present invention, a process for producing ethyl alcohol from amylaceous raw stock by its hydro-

lysis under the effect of amylolytic enzymes is characterized by that hydrolysis is carried out in the presence of an enzymic preparation of cellulase obtained from the culture of *Trichoderma kőningii*, containing a complex of hydrolytic enzymes, viz., C_1 -enzyme, endo- and exoglucanase, cellobiase, xylanase, β -glucosidase, protease, and a number of amylolytic enzymes.

The principal enzymes making part of the afore-mentioned preparation feature the following activity /in terms of units per gram/:

C_1 -enzyme/with respect to paper/	- 100 to 125
endoglucanase	- 15 to 20
exoglucanase	- 3 to 4
cellobiase	- 4 to 6
xylanase	- 200 to 250

Optimum consumption of the above preparation equals 1 to 2 per cent of the entire mass of the amylaceous raw stock involved due to the fact that in the abovesaid consumption limits more complete and economically advantageous hydrolysis is attained.

The present invention enables one to carry out deeper hydrolysis of grain polysaccharides and increase the yield of ethyl alcohol by 3 to 4 per cent as compared to the known processes. This is accounted for by the fact that the provision of such enzymes as C_1 -enzyme, endoglucanase, exoglucanase, cellobiase, and others in the proposed preparation,

are capable of hydrolyzing grain polysaccharides of the nonamylaceous nature, such as cellulose and hemicellulose, into fermentable carbohydrates, with the result that the yield of ethyl alcohol is increased and the amount of lost unfermented sugars is diminished.

The above-mentioned enzymic preparation of cellulase is obtained from cultivating a mould fungus *Trichoderma kőningii* on a solid nutrient medium, containing the following components /in terms of mass per cent/:

wheat bran	- 40 to 45
beet pomace	- 20 to 25
malt sprouts	- 25 to 30
sawdust	- 5 to 10

Cultivation is carried out at 30 to 35°C for 48 to 55 hours. On terminating the cultivation process the resultant enzymic preparation can be made use of in alcohol production or as the mould culture together with the remainder of the nutrient medium and mycelium, disintegrated and dried to a moisture content of 12 or 13 per cent; or else in the form of a preparation obtained by precipitating the enzymes from aqueous extracts of the mould fungus culture, by organic solvents.

Application of unpurified enzymic preparation in the form of the culture of the above mould fungus is economically more reasonable, as this rules out expenditures for isolating the enzymes and prevents losses of their activity in

the course of isolation.

The process of the present invention is carried out as follows. Amylaceous raw stock, e.g., wheat grains, is fed to the crusher to be disintegrated there, wherefrom the stock enters the mixer-preboiler composed of two compartments. In the first compartment the crushed grains is mixed with water in a weight ratio of 1:4, whereupon the mixture is forwarded to the second compartment, where it is heated to 80 or 85°C. While the mixture is being heated starch is rendered pastelike. Next the pastelike mass is delivered from the preboiler to the cooker arrangement, wherein the mass is cooked at a pressure of 0.4 to 0.5 MPa for 30 to 50 min. Then the cooked mass is delivered to the steam separator for getting it read of steam, and from there to the 1st-stage saccharifier to be cooled to the temperature of hydrolysis /58 to 60°C/. Fed to the saccharifier in an amount of 30 per cent ^{is} an aqueous suspension of a mixture of the hydrolytic enzymes obtained from surface cultures of mould fungi, the above suspension containing 1 per cent α -amylase from *Aspergillus oryzae* /in terms of starch mass/, 4 per cent glucoamylase from *Aspergillus awamori* /in terms of the starch mass/, and 1.5 per cent of a complex preparation of cellulase from the culture of *Trichoderma kőningii* /in terms of whole mass of raw stock/. The above aqueous suspension is treated with formalin in a 0.02 mass per cent concentration.

It is in the 1st-stage saccharifier that the cooked mass is liquefied and partially hydrolyzed under the effect of the afore-mentioned enzymes, the process proceeding at 58 to 60°C for 10 min. From the 1st-stage saccharifier the mass is transferred to the 2nd-stage saccharifier to which are added the remaining 70 per cent of the above aqueous suspension of the hydrolytic enzymes grown on surface cultures of mould fungi, whereupon starch and nonamylaceous polysaccharides undergo thorough hydrolysis in the 2nd-stage saccharifier at 57 to 58°C for 2 to 5 minutes. Next the finally hydrolyzed mass is transferred from the 2nd-stage saccharifier to the heat exchanger to be cooled to the fermentation temperature /30°C/ and from there to the fermenter vat, into which is also introduced yeast *Saccharomyces cerevisiae* XII in an amount of 6 to 8 per cent of the fermenter vat useful capacity. Thereupon the saccharified mass undergoes fermentation under the effect of the above-mentioned yeast, the fermentation process occurring at 28 to 30°C for 48 to 50 hours.

The fermentation process over, the mature yeast wash is fed to the rectifying still. A 4-per cent increase in the yield of ethyl alcohol per unit of the original amylaceous stock is attained as compared to the ethyl alcohol producing process dispensing with the proposed preparation.

The enzymic preparation of cellulose made use of for hydrolysis, is obtained by surface cultivating of a mould

fungus *Trichoderma kőningii* on a solid nutrient medium, containing the following components /in terms of mass per cent/:

wheat bran	- 45
malt sprouts	- 25
beet pomace	- 25
sawdust	- 5

The moisture content of the nutrient medium is within 60 to 65 per cent, the cultivation occurring at 30 to 35°C for 48 to 55 hours.

The finished culture of *Trichoderma kőningii* fungus is in fact an enzymic preparation of cellulase. Such preparation contains a complex of hydrolytic enzymes, mostly cellulosolytic enzymes and xylanase featuring the following activity /in terms of units per gram/:

C ₁ -enzyme /with respect to paper/	- 100 to 125
endoglucanase	- 15 to 20
exoglucanase	- 3 to 4
cellobiase	- 4 to 6
xylanase	- 200 to 250

The thus-obtained enzymic preparation of cellulase is used for hydrolysis in the form of a fungus culture "per se" without isolating and purifying with organic solvents, an advantageous feature that rules out costly and sophisticated stage of isolating the enzymes producing cellulase effect and prevents the loss of their fermenting activity

while being isolated.

To promote understanding of the present invention given below are some examples of its practical embodiment carried out under laboratory conditions.

Example 1

50 g ground wheat grains is placed in a dry Erlenmeyer flask 0.5 l in capacity, into which is added 200 ml water, whereupon the contents are stirred and rendered pastelike in a boiling water bath for 40 min. Then the flask is removed from the bath and closed with glass covers, whereupon the flask is placed in a digester where its contents are cooked at a pressure of 0.15 MPa for 90 min. After cooking the flask is removed from the digester, and added thereto for the cooked mass to liquefy is 30 per cent of a mixture of enzymes, containing the following components /in terms of per cent of starch mass/: α -amylase from *Aspergillus oryzae*, 1; glucoamylase from *Aspergillus awamori*, 4; and the cellulase preparation from *Trichoderma kőningii*, 1.5 /in terms of mass of grains/, the above preparation containing a complex of hydrolytic enzymes, i.e., C_1 -enzyme, endoglucanase, exoglucanase, cellobiase, xylanase, β -glucosidase, protease, and a number of amylolytic enzymes. The mass is cooled to 58°C, and the rest of the mixture of enzymes /70 per cent/ is added thereto, whereupon the mass is subjected to hydrolysis at the above-mentioned temperature for an hour.

After the hydrolysis the flask contents are cooled to 30°C and doped with a suspension of yeast *Saccharomyces cerevisiae* XII /6 per cent of the mass/, whereupon some formalin /0.02 per cent of the mass/ is added for sterility. Then the flask is closed and placed in a temperature-controlled cabinet for fermentation, which occurs at 30°C within 72 hours. Next ethyl alcohol is distilled from the resultant yeast wash in a distiller.

The yield of ethyl alcohol equals 19.23 ml from 50 g wheat containing 56.4 per cent starch, i.e. 104 per cent.

Used in Example 1 is the preparation of cellulase featuring the following activity of the basal enzymes /in terms of units per gram/:

C ₁ -enzyme /with respect to paper/	- 125
endoglucanase	- 17
exoglucanase	- 3
cellobiase	- 5
xylanase	- 210

The above preparation is obtained by cultivating a mould fungus *Trichoderma kőningii* on a solid nutrient medium, containing the following components /in terms of mass per cent/:

wheat bran	- 45
malt sprouts	- 25
beet pomace	- 25
sawdust	- 5

The thus-obtained fungus culture is used in a native state, i.e., without isolating the enzymes from the nutrient medium and their purifying, which to a great extent simplifies production and utilization of the preparation.

Example 2

Wheat grains /in an amount of 1000 kg/ are continuously fed to the grain crusher to be disintegrated there, whereupon the crushed grains are fed to the mixer-preboiler, composed of two compartments. Water is also fed to the 1st compartment in an amount four times the grain mass. Preliminary heat treatment of grains occurs in the 1st compartment, whereas final heat treatment of grains, i.e., rendering them pastelike, is carried out in the 2nd compartment at 80 to 85°C for 2 to 5 minutes. For final heat treatment of the pastelake mass it is fed to the cooker arrangement to be cooked at a pressure of 0.5 MPa for 50 minutes. Thereupon the mass is transferred to the steam separator for getting the mass read of steam, wherefrom the mass is forwarded to the 1st-stage saccharifier. On cooling the mass to 58 or 60°C added thereto is an aqueous suspension /30 per cent of the whole amount/ of a mixture of hydrolytic enzymes of surface cultures of mould fungi, containing 1 per cent α -amylase from *Aspergillus oryzae*, 4 per cent glucoamylase from *Aspergillus awamori* /both in terms of starch mass/, and 1.5 per cent /in terms of preparation mass/ of the cellulase preparation obtained from the culture

of a mould fungus *Trichoderma kőningii* of the composition as in Example 1. Then the aqueous suspension is treated with an antiseptic, say, formalin in a concentration of 0.02 mass per cent. The process of hydrolysis in the 1st-stage saccharifier takes 10 minutes, whereupon the mass is transferred to the 2nd-stage saccharifier to which is added the remaining 70 per cent of the above aqueous suspension of the hydrolytic enzymes of surface cultures. It is in the 2nd-stage saccharifier that starch and nonamylaceous polysaccharides undergo thorough hydrolysis at 57 to 58°C for 5 minutes. Hydrolysis over, the mass is let cool to 30°C in a heat exchanger and transferred to the fermenter vat, to which is added yeast *Saccharomyces cerevisiae* XII /6 to 8 per cent of the fermenter useful capacity/, where the mass is fermented at 28 to 30°C for 72 hours. The fermentation process over, the mature yeast wash is fed to the rectifying still to obtain ethyl alcohol therefrom. The yield of ethyl alcohol per ton of starch equals 66.56 dal, i.e., by 4 per cent higher as compared to the control process carried out according to the known method, that is, without the proposed preparation. An increased ethyl alcohol yield is attained due to fermenting the carbohydrates resulting from hydrolysis of the nonamylaceous portion of corn grains.

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The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:-

1. A process for producing ethyl alcohol from amylaceous raw stock by virtue of hydrolysis of said raw stock under the effect of amylolytic enzymes and an enzymic preparation of cellulase obtained from the culture of a mould fungus *Trichoderma kōnigii* and containing a complex of hydrolytic enzymes, viz., C₁-enzyme, exo-glucanase, endoglucanase, cellobiase, xylanase, β -glucosidase, protease, and a number of amylolytic enzymes.

2. A process as claimed in Claim 1, wherein said enzymic preparation is taken in an amount of 1 to 2 mass per cent.